Investigation of the Toxic & Teratogenic Effects of GRAS Substances to the Chicken Embryo-Report of the in-house investigations of **Zinc Gluconate** in developing chicken embryo 3/1/78

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MEMORANDUM

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION

TO : GRAS Review Branch, HFF-335 DATE: March 1, 1978

THRU: HFF-150

FROM : Supervisory Chemist
Whole Animal Toxicology Branch (HFF-155)

SUBJECT: Investigation of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo

Attached is the report of the inhouse investigations of Zinc Gluconate in the developing chicken embryo.

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Investigations of the Toxic and Teratogenic Effects of GRAS Substances to the Developing Chicken Embryo: Zinc Gluconate

Protocol:

Zinc Gluconate (1) was tested for toxic and teratogenic effects to the developing chicken embryo under four sets of conditions. It was administered in water as the solvent by two routes and at two stages of embryonic development; via the air cell at pre-incubation (0 hours) and at 96 hours of incubation, and via the yolk at 0 hours and at 96 hours using techniques that have been described previously (2,3).

Groups of fifteen or more eggs were treated under these four conditions at several dose levels until a total of seventy-five to one hundred eggs per level was reached for all levels allowing some to hatch.

Groups of comparable size were treated with the solvent at corresponding volumes and untreated controls were also included in each experiment.

After treatment, all eggs were candled daily and non-viable embryos removed. Surviving embryos were allowed to hatch. Hatched chicks and non-viable embryos were examined grossly for abnormalities (internally and externally) as well as for toxic responses such as edema and hemorrhage. All abnormalities were tabulated.

Results:

The results obtained are presented in Tables 1 through 4 for each of the four conditions of test.

Columns 1 and 2 gave the dose administered in milligrams per egg and milligrams per kilogram, respectively. (The milligrams per kilogram figure is based on an average egg weight of fifty grams.)

Column 3 is the total number of eggs treated.

Column 4 is the percent mortality, i.e., total non-viable divided by total treated eggs.

Column 5 is the total number of abnormal birds expressed as a percentage of the total eggs treated. This includes all abnormalities observed and also toxic responses such as edema, hemorrhage, hypopigmentation of the down and other disorders such as feather abnormalities, significant growth retardation, cachexia or other nerve disorders.

Column 6 is the total number of birds having a structural abnormality of the head, viscera, limbs, or body skeleton expressed as percentage of the total eggs treated. Toxic responses and disorders such as those noted for column 5 are not included.

Column 3 through 6 have been corrected for accidental deaths if any occurred. Included in these columns are comparable data for the solvent-treated eggs and untreated controls.

The mortality data in column 4 have been examined for a linear relationship between the probit percent mortality versus the logarithm of the dose according to the procedures of Finney (4). The results obtained are indicated at the bottom of each table.

The data of columns 4, 5 and 6 have been analyzed using the Chi Square test for significant differences from the solvent background. Each dose level is compared to the solvent value and levels that show differences at the 5% level or lower are indicated by an asterisk in the table.

Discussion:

Air cell treatment with zinc gluconate resulted in low toxicity at 0 hours and moderately high toxicity at 96 hours. The calculated LD_{50} s are as follows:

Air cell at 0 hours 196.3 mg/kg (9.815 mg/egg)
Air cell at 96 hours 7.70 mg/kg (0.385 mg/egg)

Yolk treatment at both times of administration resulted in low toxicity and no LD_{50}s could be calculated in the range tested.

Scattered abnormalities were observed under all conditions of test, but serious abnormalities were in no instance significantly higher than or different from those observed in the background. Zinc gluconate displayed no teratogenicity under the test conditions employed.

1. Zinc Gluconate, Ruger Chemical Co. Irvington, NJ, Lot #Y4753H29

2. McLaughlin, J., Jr., Marliac, J. P., Verrett, M. Jacqueline, Mutchler, Mary K., and Fitzhugh, O.G., (1963) Toxicol. Appl. Pharmacol 5, 760-770

3. Verrett, M. J., Marliac, J. P., and McLaughlin, J., Jr., (1964) JAOAC 47, 1002-1006

4. Finney, D. J., (1964) Probit Analysis, 2nd Ed., Cambridge Press, Cambridge, Appendix I.

ZINC GLUCONATE
AIR CELL AT 0 HOURS

TABLE 1

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	110	59.09*	6.36	3.63
5.00	100.00	110	46.36*	3.63	1.81
2.50	50.00	110	21.81	1.81	0.00
1.250	25.00	109	25.68*	1.83	0.00
0.50	10.00	109	26.60*	1.83	0.91
Water		109	12.84	2.75	0.00
Control		345	10.43	1.15	1.15

*Significantly different from solvent $p \leq 0.05$

**LD_{50} 196.298 mg/kg (9.815 mg/egg)

ZINC GLUCONATE
AIR CELL AT 96 HOURS

TABLE 2

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
5.00	100.00	30	100.00*	0.00	0.00
2.50	50.00	115	100.00*	0.00	0.00
1.250	25.00	115	93.04*	0.00	0.00
0.6250	12.50	115	73.91*	6.95	4.34
0.250	5.00	115	48.69*	2.60	2.60
0.1250	2.50	115	32.17	7.82	3.47
WATER		104	30.76	3.84	2.88
CONTROL		345	10.43	1.15	1.15

*Significantly different from solvent $p \leq 0.05$

**LD_{50} 7.702 mg/kg (0.385 mg/egg)

ZINC GLUCONATE
YOLK AT 0 HOURS

TABLE 3

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
10.00	200.00	110	64.54*	0.00	0.00
5.00	100.00	110	61.81*	0.00	0.00
2.50	50.00	109	60.55*	1.83	1.83
1.250	25.00	109	60.55*	0.00	0.00
0.500	10.00	110	54.54*	0.00	0.00
WATER		115	26.08	0.86	0.86
CONTROL		345	10.43	1.15	1.15

*Significantly different from solvent $p \leq 0.05$

**Slope not significantly different from zero $p = 0.05$

ZINC GLUCONATE
YOLK AT 96 HOURS

TABLE 4

Dose mg/egg	Dose mg/kg	Number of Eggs	**Percent Mortality	Percent Abnormal Total	Percent Abnormal Structural
5.00	100.00	80	52.50*	8.75	7.50
2.50	50.00	105	48.57*	5.71	5.71
1.250	25.00	105	54.28*	4.76	0.95
0.6250	12.50	105	41.90	3.80	0.95
0.250	5.00	105	43.80	1.90	1.90
0.1250	2.50	25	60.00*	12.00	8.00
Water		105	33.33	2.85	0.95
Control		345	10.43	1.15	1.15

*Significantly different from solvent $p \leq 0.05$

**Slope not significantly different from zero $p = 0.05$